

Test Kit Instruction

August 5, 2016

Charm Sciences, Inc.
ROSA WET-S3 Aflatoxin Quantitative Test

FORWARD

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the Mycotoxin Handbook for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

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Contents

1. GENERAL INFORMATION..... 3

2. PREPARATION OF TESTING MATERIALS AND EQUIPMENT..... 4

3. EXTRACTION PROCEDURES..... 5

4. SAMPLE PREPARATION FOR QUANTITATION..... 6

5. TEST PROCEDURES 6

6. REPORTING AND CERTIFYING TEST RESULTS..... 8

7. STORAGE CONDITIONS AND PRECAUTIONS..... 9

8. EQUIPMENT AND SUPPLIES 10

9. REVISION HISTORY..... 11

10. FLOW CHART 12

1. GENERAL INFORMATION

ROSA WET-S3 Aflatoxin Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology and Water Extraction Technology (WET) that eliminates the use of organic solvents. WET uses a non-hazardous extraction powder added to the sample followed by water to extract aflatoxins into the aqueous solvent. Aflatoxins interact with colored beads in the lateral flow test strip and the color intensity in the test zone and control zone is measured by the Charm EZ-M reader and interpreted as parts per billion (ppb) aflatoxins.

Approved Test Kit Information	
Test Kit Vendor:	Charm Sciences, Inc. 978-687-9200
Test Kit Name:	ROSA WET-S3 Aflatoxin Quantitative Test
Product Number:	LF-AFQ-WETS3
Effective Date of Instructions:	08/05/2016
Instructions Revision Number:	0
Conformance Range:	5.0 ppb to 300 ppb
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Approved Commodities:	Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings)
Extraction method:	Shake vigorously by hand for 1.5 minutes.
Test Format:	Lateral flow strip
Detection Method:	Charm EZ-M reader, Model LF-ROSA-EZ-M

2. PREPARATION OF TESTING MATERIALS AND EQUIPMENT

a. Test Strips.

Remove from the container only the number of test strips to be used in 1 day. Keep these test strips at room temperature (18 °C to 30 °C) during daily use for up to 12 hours; discard the unused test strips after the 12-hour period.

b. AFQ Dilution Buffer.

(1) Dispense buffer into a clean micro-centrifuge tube and label for each sample to be tested.

(2) Use pre-dispensed buffer tubes and buffer solution at room temperature.

c. EP Control.

(1) Prepare EP Control by dissolving one packet of WET-S Extraction Powder in 150 milliliters (mL) deionized or distilled water by gently swirling until extraction powder is dissolved.

(2) Use to prepare Negative Control and Positive Control.

d. Negative Control.

(1) Prepare Negative Control by adding 300 microliters (µL) EP Control to 600 µL AFQ Dilution Buffer in a clean micro-centrifuge tube, cap, mix, and label.

(2). Use Negative Control in TEST PROCEDURES section.

e. Positive Control.

(1) Reconstitute the dry Positive Control by adding 1.0 milliliter (mL) EP Control followed by 3.0 mL AFQ Dilution Buffer. Cap, shake well and allow to stand at room temperature for 10 minutes. Mix before use.

(2) Use reconstituted Positive Control in TEST PROCEDURES section.

f. Reader and Strip Performance Check.

(1) Enter performance mode in Charm EZ-M reader by selecting Perf. Mon. from the Main Menu, followed by Perf. Test.

(a) Follow the system prompts to test calibration strips (LO CAL and HI CAL).

(b) Follow the system prompts to test controls (NEG CTRL and POS CTRL); select AFQ-WETS3 from the TESTS list if prompted.

- (2) Test calibration strips daily to verify Charm EZ-M reader performance. Calibration strips must test/perform in the specified ranges.
- (3) Test Negative Control and Positive Control weekly to verify test strip performance. Valid control ranges are:
 - (a) Negative Control: less than or equal to 2 ppb
 - (b) Positive Control: 12 ppb to 28 ppb

If calibration strips or controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance. Notify your monitoring field office or TSD with any documented information for quality control purposes.

g. ROSA Incubator.

- (1) ROSA Incubator must be clean and level.
- (2) The ROSA Incubator temperature must be at $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (the temperature indicator should match the incubator temperature).

3. EXTRACTION PROCEDURES

Procedure for Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings).

- a. Weigh 50 ± 0.2 grams ground sample into a clean extraction container.
- b. Add contents of one packet of WET-S Extraction Powder.
- c. Add 150 mL deionized or distilled water.
- d. Shake vigorously by hand for 1.5 minutes.
- e. Transfer 1 mL to 1.5 mL extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (centrifuge within 30 minutes of extraction and use within 2 hours).

4. SAMPLE PREPARATION FOR QUANTITATION

- a. Preparation of Diluted Extract for 5.0 ppb to 100 ppb quantitation.
 - (1) Pipet 600 µL AFQ Dilution Buffer into a clean micro-centrifuge tube.
 - (2) Pipet 300 µL centrifuged extract to micro-centrifuge tube containing 600 µL AFQ Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This sample is the Diluted Extract.
 - (3) Repeat for additional samples.
 - (4) Use Diluted Extract (use within 6 hours of preparation) as your test sample in Sample Analysis found in TEST PROCEDURES section.
- b. Preparation of Second Diluted Extract for 100 ppb to 300 ppb quantitation.
 - (1) Pipet 1000 µL AFQ Dilution Buffer into a clean micro-centrifuge tube.
 - (2) Pipet 100 µL Diluted Extract to micro-centrifuge tube containing 1000 µL AFQ Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This sample is the Second Diluted Extract.
 - (3) Repeat for additional samples.
 - (4) Use Second Diluted Extract (use within 6 hours of preparation) as your test sample in Sample Analysis found in TEST PROCEDURES section.

5. TEST PROCEDURES

- a. Sample Analysis.
 - (1) Check that the ROSA Incubator temperature is $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
 - (2) Label test strip(s) to identify sample.
 - (3) Place test strip in the ROSA Incubator with the flat side facing upward.
 - (4) Hold the test strip flat in the ROSA Incubator and use tab to expose sample compartment by peeling tape back to "Peel to Here" line. Avoid lifting the test strip and sponge under tape and bending back the white wick and sponge under the tape.
 - (5) Holding pipet vertically, slowly pipet 300 µL test sample (diluted extract or control) into the sample compartment at the ROSA Incubator line.
 - (6) Reseal the tape over the sample pad compartment.

When performing two test strips using a ROSA Incubator:

- (a) Peel, pipet, and reseal before starting next strip.
- (b) Complete all test strips within 30 seconds.
- g. Close lid on the ROSA Incubator.
- h. Incubate for 3 minutes.
- i. Remove strip from the ROSA Incubator.

Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted.

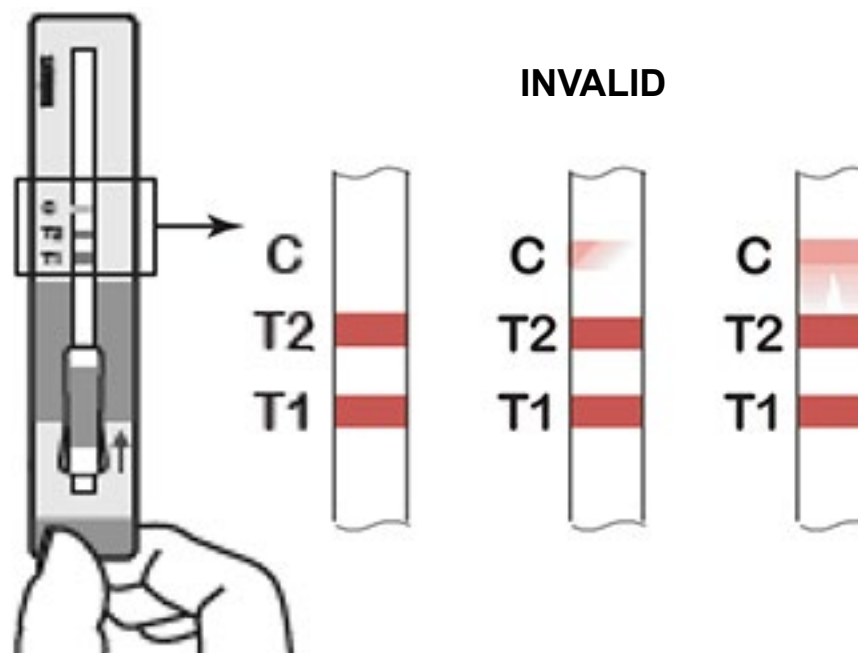
- (1) Wipe foreign matter (dust, etc.) from the test strip.
- (2) Inspect and interpret test strip.

When running multiple test strips in the ROSA Incubator, remove one strip for visual inspection and interpretation at a time and complete procedure for all test strips within 1 minute of incubation completion.

- (3) Lower ROSA Incubator lid; do not re-latch.

b. Visual Inspection.

- (1) The test strip is INVALID if any of the following are observed:
 - (a) C (Control) line is missing.
 - (b) T1, T2 (Test) or C line is smeared or uneven.
 - (c) T1, T2, or C line is obscured by diluted extract or control.
 - (d) Beads do not flow past T1, T2 or C lines.



- (2) Do not put INVALID test strips in the Charm EZ-M reader.
- (3) If test strip is INVALID, re-test the diluted extract or control.

c. Interpretation.

- (1) Insert a clean and valid test strip into the Charm EZ-M reader. Slide the strip into the slot with the sample compartment in the down position until it stops.
- (2) Read results on AFQ-WETS3 from the TESTS list with CORN (COMMODITY) and DILUTION selected for sample. If desired, enter OPERATOR ID, SAMPLE ID, and/or LOT NUMBER. Close door to read.
 - DE: Diluted Extract for 5.0 ppb to 100 ppb quantitation.
 - 2ND DE: Second Diluted Extract for 100 ppb to 300 ppb quantitation.

Note: For controls, see Reader and Strip Performance Testing in PREPARATION OF TESTING MATERIALS AND EQUIPMENT section.

- (3) READING: The number displayed is the concentration of aflatoxins (ppb) in the sample.

A Diluted Extract READING greater than 100 ppb indicates that the sample concentration is greater than the sensitivity range of the sample dilution; prepare Second Diluted Extract and perform assay with another test strip.

A Second Diluted Extract READING less than 53 ppb indicates a value below the sensitivity range of the sample dilution; perform assay with another test strip using Diluted Extract.

A Second Diluted Extract READING greater than 300 ppb indicates that the sample concentration is greater than the sensitivity range of the sample dilution; report test result as greater than 300 ppb on the work record and certify as "Aflatoxins exceed 300 ppb".

6. REPORTING AND CERTIFYING TEST RESULTS

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

7. STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions.

- (1) Store test strips refrigerated (0 °C to 7 °C) in tightly-closed supplied container.
- (2) Store dilution buffer bottle and pre-dispensed micro-centrifuge tubes refrigerated.
- (3) Store WET-S Extraction Powder at room temperature in supplied packet.
- (4) Store EP Control refrigerated for up to 1 week or aliquot (at least 1.5 mL) to clean micro-centrifuge tubes, label, and freeze (15 °C or below) within 6 hours of reconstitution for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed EP Control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.
- (5) Store dry Aflatoxin B1 Positive Control refrigerated.
- (6) Store reconstituted Positive Control refrigerated for up to 1 week or aliquot (at least 0.5 mL) to clean micro-centrifuge tubes, label, and freeze within 6 hours of reconstitution for up to two months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed Positive Control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.

b. Precautions.

(1) Test Strips.

- (a) To open test strip canister, remove and save plastic lid with foil-lined foam insert to reseal container, lift foil tab, and peel foil seal off container. Discard foil seal.
 - (b) In high humidity, limit condensation by opening container after it has warmed to room temperature.
 - (c) Inspect/verify desiccant indicator. Beads inside desiccant packets should be blue. Discard test strips if the blue beads have turned purple or pink.
 - (d) Re-shape dented sample compartments to fit into ROSA Incubator.
- (2) Use AFQ Dilution Buffer supplied with each test kit only at room temperature. Keep buffer at room temperature during daily use for up to 12 hours.
 - (3) WET-S Extraction Powder is non-hazardous and may be disposed as normal waste. Do not open WET-S Extraction Powder until ready to use.

- (4) Do not use the test kits beyond the noted expiration date.
- (5) Debris on test strips may alter the reader optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into reader.
- (6) ROSA Incubator must be clean and level. ROSA Incubator temperature must be $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The temperature indicator should match the ROSA Incubator temperature. A daily thermometer check is recommended. Keep ROSA Incubator lid lowered, but not latched, unless performing test procedure. ROSA Incubator may take 10 minutes to reach proper temperature depending on ambient temperature.
- (7) Charm EZ-M reader must be clean and level. Keep reader lid closed unless performing procedure.

8. EQUIPMENT AND SUPPLIES

a. Materials provided in test kits.

(1) Test Strips.

(a) LF-AFQ-WETS3-20K/-20ESK.

- 1 One container of 20 AFQ-WETS3 test strips
- 2 Aflatoxin B1 Positive Control(s):
 - a One control in LF-AFQ-WETS3-20K
 - b Two controls in LF-AFQ-WETS3-20ESK
- 3 One AFQ Dilution Buffer

(b) LF-AFQ-WETS3-100K/-100ESK.

- 1 One container of 100 AFQ-WETS3 test strips
- 2 Aflatoxin B1 Positive Control(s):
 - a One control in LF-AFQ-WETS3-100K
 - b Five controls in LF-AFQ-WETS3-100ESK
- 3 One AFQ Dilution Buffer

(c) LF-AFQ-WETS3-500K/-500ESK.

- 1 Five containers of 100 AFQ-WETS3 test strips
- 2 Aflatoxin B1 Positive Controls:
 - a Five controls in LF-AFQ-WETS3-500K
 - b Twenty-five controls in LF-AFQ-WETS3-500ESK
- 3 Five AFQ Dilution Buffers

(2) WET-S Extraction Powder.

- (a) LF-WET-EXTS-50G-20: WET-S Extraction Powder for 50 gram sample (20/pack)
- (b) LF-WET-EXTS-50G-100: WET-S Extraction Powder for 50 gram sample (100/pack)

b. Materials required but not provided.

- (1) 100 µL pipet and pipet tips
- (2) 300 µL pipet and pipet tips
- (3) 1000 µL fixed volume pipet or 100 µL to 1000 µL variable volume pipet and pipet tips
- (4) 250 mL graduated cylinders
- (5) Balance
- (6) Charm EZ-M reader
- (7) Deionized or distilled water
- (8) Micro-centrifuge tubes
- (9) Mini-centrifuge
- (10) Printer for Charm EZ-M reader (optional)
- (11) ROSA Incubator
- (12) Sample extraction containers or Whirl-pak bags
- (13) Sample grinder
- (14) Transfer pipets

9. REVISION HISTORY

Revision 0 (08/05/2016)

10. FLOW CHART

Refer to GIPSA Test Kit Instructions for Complete Test Procedure

ROSA® WET®-S3 Aflatoxin Quantitative Test Flow Chart

Approved Commodity:
Corn

See Approved Commodity Below

Quantitation Ranges: 5.0 to 100 ppb

Sample Preparation

(1) Weigh
Ground sample
50.0 ± 0.2 g

(2) Add WET-S Extraction Powder
1 Packet for 50 g sample

(3) Add Solvent
Deionized or Distilled Water
150 mL

(4) Extract
Shake vigorously for 1.5 minutes

(5) Clarify
Centrifuge extract for 10 seconds

(6) Dilute
Prepare Diluted Extract.

Test Procedure

(1)
Place test strip in ROSA Incubator.

(2)
Peel tape.
Pipet 300 µL Diluted Extract into sample compartment.
Reseal tape.

(3)
Close lid.
Incubate for 3 minutes.

Read Result

(1) Inspect test strip

(2) Read result with Charm EZ®-M reader

Select appropriate test (AFQ-WETS3), commodity (CORN) and dilution if prompted.

Valid Test

Invalid Test

Sample	Dilution	Quantitation Range
Diluted Extract	DE	5.0 to 100 ppb
2 nd Diluted Extract	2ND DE	100 to 300 ppb

For quantitation of 100 to 300 ppb:

(1) Prepare 2nd Diluted Extract

(2) Repeat Test Procedure (steps 1, 2, 3) with 2nd Diluted Extract

(3) Read Results

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